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Preliminary Observations on the Effects of Treflan to Fishes

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Preliminary observations on the effects of Treflan to fishes

(TITLE)

BY

P. Bradley Latvaitis

THESIS

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ABSTRACT

Treflan E.C. is a selective, preemergence, soil incorporated herbicide. Preliminary tests were made to evaluate its effects at concentrations of 0.75, 1.275 and 1.8 p.p.m. on twenty species of fresh water fishes in the laboratory. The herbicide was proven to be a powerful fish toxicant. Centrarchus, Cichlasoma centrarchus, was the most resistant fish tested, while Bluegill, Lepomis macrochirus, was among the least resistant. Treflan E.C. decomposes and degrades rapidly. It was also found to interact with plastics. Further studies are necessary to provide a complete investigation of the effects of Treflan on Fishes.

INTRODUCTION

Due to the practical difficulty of simulating natural conditions, it is difficult to determine the specific toxicity of a given material. Laboratory experiments, such as the bioassay, give valuable guidance in determining the concentrations of materials that will seriously affect fish under laboratory conditions. These results can then be the basis for investigation of the effects of a substance, such as Treflan, to fishes in natural environments.

Bliss (1957) defined a bioassay as a determination of the potency of a physical, chemical, or biological agent by means of a biological indicator. Noting its development during the past 20 or 30 years by scientists from many diverse fields, he listed the principles which characterize the modern bioassay: 1) potency is a property of the drug, not of the response; 2) potency is relative, not absolute; 3) the assayed potency of an unknown is only an estimate of its true value; and 4) both the reliability and efficiency of an assay are linked inseparably with its design. These principles must be observed to use the bioassay as an effective research tool.

The observations on the toxicity of Treflan to twenty species of fishes provides fundamental data on the effects of Treflan on fish. The bioassay also provides preliminary data on the sensitivities of the families of fishes that were tested.

TREFLAN

Treflan, the registered trademark name for a,a,a-trifluoro-2, 6-dinitro-N,N-dipropyl-p-toluidine (Elanco Products Company, 1968) is more commonly known by the generic name trifluralin. It is a selective, preemergence, soil incorporated herbicide controlling a wide variety of grasses and broadleaf weeds. Trifluralin is being widely used for control of weeds in cotton (Gossipium hirsutum L.) and soybeans (Glycine max L.). It is normally applied in the spring and incorporated up to six weeks before planting. Treflan E.C. (emulsifiable concentrate containing 44.5% active ingredients and 55.5% inert ingredients) is mixed with 5 to 40 gallons of water and applied according to broadcast rates which are specific for soil type and amount of rainfall.

Mechanisms of Action

Several studies, at the tissue level, have shown Treflan effectively inhibits root growth (Feeny 1966, Fischer 1966, Lignowski and Scott, 1972a). The major histological studies indicate trifluralin affects mitosis in roots (Talbert 1965, Amato et al. 1965, Bayer et al. 1967). Lignowski and Scott (1972b) showed that as soon as prophase proceeded to metaphase, mitosis became inhibited, and the later stages of mitosis did not occur. The chromosome aberrations produced by trifluralin are characteristic of colchicine action, the classic spindle disrupting agent (Eigsti and Dustin, 1955). The major morphological characteristic is the swelling of root terminals caused by changes in the polarity of growth of elongating cortical cells (Amato et al. 1965, Bayer et al. 1967, Lignowski and Scott 1972a).

Little has been published concerning trifluralin's effect on other physiological and biochemical processes. Negi et al. (1968), showed that both oxygen uptake and oxidative phosphorylation ratios were reduced by trifluralin in isolated mitochondria. It is not certain that such an inhibition would take place in intact plants. The inhibitory activity of trifluralin, when compared with a respiratory inhibitor, sodium azide, showed both chemicals were equally inhibitory to oxygen uptake. It may be concluded that inhibition of these processes could be involved in the mechanism of action of the herbicide.

Preliminary reports indicate inhibition of the photosynthetic process as not a mechanism of action for Treflan (Negi et al., 1968).

Chemical and Physical Characters

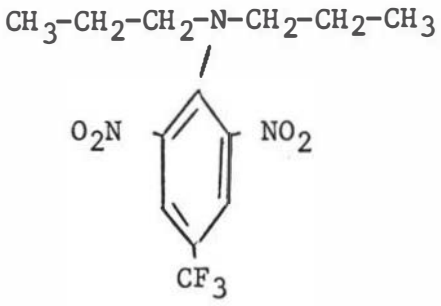

Figure 1 - Structure of Trifluralin

Formula: $C_{13}H_{16}F_3N_3O_4$ M.W. 335.28

Melting point: 48.5-49°C

Boiling point: 96-97°C at 0.18 mm H_g

Solubility in water: Less than 1.0 ppm at 27°C

Soluble in: organic solvents such as methanol, acetone, xylene and chloroform

(Probst et al. 1967)

Experimentation by Wright and Warren (1965) reported the influence of sunlight and artificial light on the chemical characteristics and biological activity of trifluralin. It was concluded that light absorbed by trifluralin in the 360-380 millimicron band may provide sufficient energy to cause degradation of the compound. Direct laboratory bioassays of trifluralin exposed to sunlight on glass show all biological activity is lost after six hours exposure. Field studies of trifluralin exposed to sunlight on soil show some herbicidal activity remained after six hours. This is due to the uneven surface the soil presents which alters direct radiation. Soil adsorption, soil moisture, and pH complicate the availability of the compound.

Soil to which trifluralin has been applied has been shown to emit vapors that can be collected in xylene and assayed by gas chromatography (Swann and Behrens, 1972a). Additional studies by Swann and Behrens (1972b), show trifluralin vapors arising from soil inhibited growth of roots and shoots of foxtail millet (Setaria italica L.) and proso millet (Panicum miliaceum L.) and become lethal at increased concentrations.

Treflan is strongly absorbed onto the soil particles and is extremely resistant to movement by water. During heavy rainfall Treflan is not leached from the weed seed germination zone which is located 2-6 inches below the surface of the soil (Elanco Products Company, 1968). Calculations by Parka and Worth (1965) show that an inconceivably high amount of soil erosion would be necessary to introduce toxic concentrations of trifluralin into a pond or stream when applied according to label instruction. Trifluralin is properly applied when sprayed on the soil surface with low-pressure, low-volume equipment as prescribed by broadcast rates for the area of incorporation.

Immediate incorporation into the soil with equipment adjusted to cut to a depth of 2-4 inches follows spraying.

Aerobic Degradation: A survey by Elanco Products Company (1968) showed trifluralin did not accumulate in soils treated for up to four consecutive years, when used according to label instructions. Studies reported by Probst et al. (1967) demonstrated trifluralin continuously degraded in the soil. Less than 50 parts per billion of trifluralin was found in most soils about 160 days after suggested application. The postulated pathway of aerobic trifluralin degradation (aerobic condition being defined as soil condition of normal exposure to light, atmosphere and moisture) is demonstrated in Figure 2. Intermediates, corresponding to model compounds, were detectable only in small amounts, suggesting rapid conversion to mixtures of polar products after the initial degradation step to a,a,a-Trifluoro-2,6-dinitro-N-propyl-p-toluidine. This initial degradation product observed in field soil is also the initial step in photodecomposition. It serves as an intermediate for a dual pathway of decomposition.

The persistence of trifluralin increases with depth of soil incorporation due to (a) decrease of dissipation due to photodecomposition, (b) microbiological and chemical decomposition affected by variation in aeration, soil moisture and temperature and (c) volatilization losses due to less rapid gas exchange and lower temperatures below the surface (Savage and Barrentine, 1968).

Anaerobic Degradation: Further investigations by Probst et al. (1967) involved degradation of Treflan under anaerobic conditions. Persistence of

trifluralin under moisture contents equivalent to 200% field capacity showed the herbicide was lost very rapidly. Fifty per cent had disappeared within ten days and 84% disappeared in 24 days. The rate of breakdown was not influenced markedly by soil type. It was concluded that when soil is supersaturated with water, trifluralin begins to degrade immediately by the aerobic pathway (Figure 2) until the oxygen in the system is depleted.

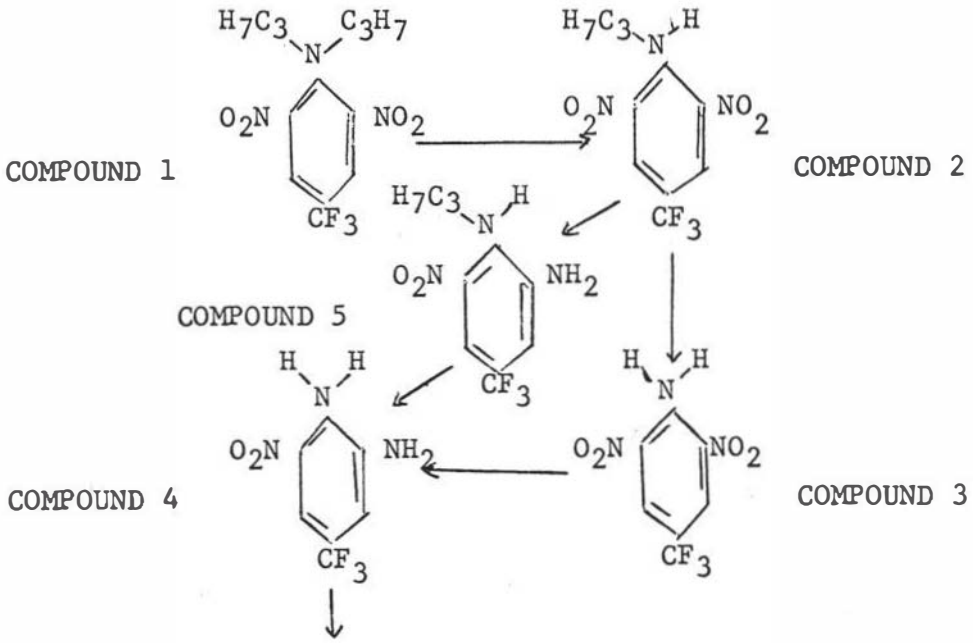

Figure 2. Postulated pathway of aerobic trifluralin degradation

Compound #1 Trifluralin

Compound #2 a,a,a-Trifluoro-2,6-dinitro-N-propyl-p-toluidine

Compound #3 a,a,a-Trifluoro-2,6-dinitro-p-toluidine

Compound #4 a,a,a-Trifluoro-5-nitrotoluene-3,4-diamine

Compound #5 a,a,a-Trifluoro-5-nitro-N^4-propyltoluene-3,4-diamine

Twelve hours after supersaturation very small quantities of a,a,a-Trifluoro-2,6-dinitro-N-propyl-p-toluidine, the major product of the aerobic pathway, is present. Thereafter, the major degradation product is a,a,a-Trifluoro-N^4,N^4-dipropyl-5-nitrotoluene-3,4-diamine which is the major reduction product of anaerobic degradation. The maximum amount of this derivative appears during the fifth or sixth day following supersaturation and is a rate-limiting reaction. The conversion of this compound to polar products is very rapid and constitutes the major role of decomposition. The anaerobic pathway is illustrated in Figure 3.

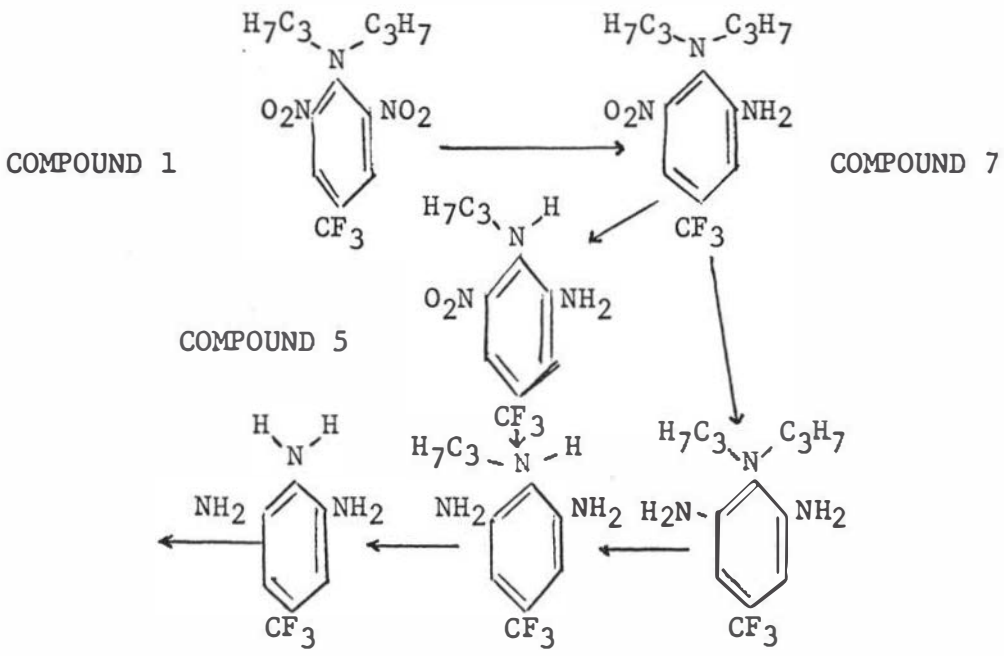

Figure 3. Postulated pathway of anaerobic trifluralin degradation

Compound #1 Trifluralin

Compound #5 a,a,a-Trifluoro-5-nitro-N^4-propyltoluene-3,4-diamine

Compound #7 a,a,a-Trifluoro-N^4,N^4-dipropyl-5-nitrotoluene-3,4-diamine

Compound #9 a,a,a-Trifluoro-N^4,N^4-dipropyltoluene-3,4,5-triamine

Compound #26 a,a,a-Trifluorotoluene-3,3,5-triamine

Compound #31 a,a,a-Trifluoro-N^4-propyltoluene-3,4,5-triamine

Bioassay Investigations

Concern has been expressed as to the safety of trifluralin to fish. The Fish and Wildlife Service in Denver, Colorado reported Treflan E.C. as one of the most toxic herbicides ever tested on fish. Their LC_{50} values (Lethal Concentration causing 50% mortality) determined in standard laboratory water toxicity test ranged between .12-.40 parts per million for rainbow trout fingerlings and .18-.70 parts per million for bluegill fingerlings. Additional experimentation by Elanco Products Company (1968) provided the following data: static water tests (procedures suitable to detect and evaluate toxicity that is not associated with excessive oxygen demand and that is due to relatively stable substances), LC_{50} 0.58 parts per million, bluegill (Lepomis macrochirus); LC_{50} 0.94 parts per million, fathead minnow (Pimephales promelas); LC_{50} 0.59 parts per million, goldfish (Carassius auratus).

Static soil tests in which trifluralin was incorporated in soil and the soil was added to water containing fish were conducted by Parka and Worth (1965). These toxicity studies indicate what amounts of erosion of soil treated trifluralin would be necessary to cause toxicities to fish. When data was extrapolated to field conditions the results demonstrated fish could not be harmed by trifluralin exposure through erosion. It was calculated it would be necessary to move the top two inches of trifluralin incorporated Princeton Fine Sand from 45.6 acres into a one acre pond, averaging three feet in depth, to produce a LC_{50} to bluegills. Using Brookston Silty Clay 4.7 times as much soil erosion would be necessary.

In simulated pond studies Treflan E.C. was incorporated into the soil in the bottom of plastic pools at rates ranging from 0 to 16 pounds per acre prior to adding water and fish. Water analyses showed that Treflan was not released from the soil into the water at any time during the study. Normal growth and reproduction of fathead minnows was observed at all rates. The toxicological data suggests when Treflan is used according to label direction, there is no hazard to fish (Parka and Worth, 1965).

MATERIALS AND METHODS

Bioassays were conducted according to standards recommended by Doudoroff et al. (1951) and the American Public Health Association Standard Methods (1971). The bioassay laboratory was a heated and air conditioned room maintained at temperatures between 74°F and 78°F. Three one hundred watt Sylvania bulbs provided lighting 8-9 hours daily. The test containers were glass jars and glass aquaria. They were cleaned thoroughly after each 24 hour period. Soap and water followed by acetone rinsing and final rinsing with deionized water removed any toxic residues. The depth of solution in test containers was never under ten inches.

The testing chemical was Treflan E.C. provided by Elanco Products Company, Greenfield Laboratories, Division of Eli Lilly and Company, Greenfield, Indiana. Lot number T - 01019 was the source of the Treflan E.C. used in the bioassays. It was maintained in a darkened container and environment so degradation, as described by Wright and Warren (1965), would not take place. Treflan E.C. was administered at 0.75, 1.275 and 1.8 parts per million. Stock solutions containing 1.5 ml herbicide to 2.0 liters of deionized water were prepared daily. Dosages of Treflan E.C. stock solution were applied within thirty minutes following preparation to minimize degradation as described by Probst et al. (1967). It was not determined whether trifluralin or degradation products of trifluralin were responsible for the majority of the afflictions that occurred in the specimens.

Following the bioassays which continued for 96 hours, the specimens were removed from test solutions and placed in bioassay water containing no herbicide. Recovery observations were made for twenty days. The bioassay solutions were renewed at twenty-four hour intervals to maintain a more uniform concentration of herbicide.

The sources of the fishes used in the bioassay were as follows: Cichlidae - Cichlasoma centrarchus (Gill and Bransford), Haplochromis burtoni (Gunther), Pseudotropheus auratus (Boulenger); Characidae - Ctenobrycon spilurus (Cuvier and Valenciennes), Hemigrammus rhodostomus (Ahl), Hemigrammus ocellifer (Steindachner); Cyprinidae - Barbus filamentosus (Cuvier and Valenciennes), Barbus conchonius (Hamilton and Buchanan), Carassius auratus (Linnaeus); Anabantidae - Trichogaster trichopterus (Pallas); Poeciliidae - Mollienisia latipinna (Meek), Lebistes reticulatus (Peters), Xiphophorus maculatus (Gunther), Xiphophorus variatus (Meek), Xiphophorus helleri (Heckel); Callichthyidae - Corydoras aeneus (Gill), were obtained from John Graves Shedd Aquarium's reserve tanks. They represent fishes, or are progeny of fishes, purchased from the Auburndale Goldfish Company Incorporated, Chicago, Illinois. Centrarchidae - Lepomis macrochirus (Rafinesque) were obtained from the Illinois Department of Conservation and were excess fish of a University of Illinois, Circle Campus, research project. Ictaluridae - Ictalurus punctatus (Rafinesque) were purchased from the J. Morreale Game Fish Farm in Richmond, Illinois. Cyprinidae - Notemigonus crysoleucas (Mitchill) and Pimephales promelas (Girard), were purchased from an independent bait dealer in Palos Park, Illinois. These fishes were originally obtained from a Wisconsin wholesaler.

The scientific names and taxonomic information provided above, were obtained from American Fisheries Society (1970), Fryer and Iles (1972), Goldstein (1970), Innes (1966), Sterba (1966) and Gill and Bransford (1877).

To insure a relative equal hardiness of specimens taken from similar environments, the test specimens were of uniform size. The length of the largest fish was not more than 1.5 times the length of the smallest specimen. Fish were not over two-fifths the length of the test container, when a cylindrical jar, or one-fourth the sum of the length and width of a rectangular aquarium as suggested by Hart et al. (1945). At least ten fish were used to test each experimental concentration. One gram of fish per liter of test solution dictated the number of fish per test container.

To avoid build up of excrement and unconsumed food and also guard against fluctuations in metabolic rate, fishes were not fed during bioassays. They were fed Tetramin, ground smelt and horseheart daily up to two days before the 96 hour bioassay. Fishes received food daily during the twenty day recovery period. They were fed sparingly during recovery. Any excrement or unconsumed food that accumulated was removed by making a partial water change. Concurrent control tests were performed with each series of bioassay observations. Conditions prescribed for bioassays were followed exactly with the exception that control solutions contained experimental water without herbicide. Control deaths were under 10%. Specimens that were not obtained from the John Graves Shedd Aquarium reserve tanks had to be transported to the Aquarium. Transportation of fish involved many considerations. When fish are excessively stimulated, as in the case of handling and transport, their metabolic rate rises to its probable maximum, which

is four or five times as great as their minimal metabolic rate, creating a greater oxygen demand and increasing the out-put of metabolic wastes (Lewis, 1963). Therefore fishes undergoing transportation received artifical aeration and were removed from the transportation water as soon as possible. As described by Lewis (1963) one pound of fish to three or four gallons of water was the rule when transporting fish. This rule is necessary because crowding may cause an increase in carbon dioxide concentration which interferes with the fishes' utilization of oxygen and causes an increase in the water's pH. Fish exhibit signs of anesthesia at 40 to 50 ppm carbon dioxide. McFarland and Norris (1958) demonstrated delayed mortality that appeared to be associated with concentrations even below twenty ppm.

Following transportation to the bioassay laboratory, the fishes were acclimatized to control conditions. Acclimatization was accomplished by providing sufficient time for the fishes to become physiologically adjusted to any great changes in their new environment. Temperature acclimatization was accomplished by allowing the transportation water temperature to become equal to the control temperature by submerging a plastic bag containing the new arrivals into control water. All animals used in bioassays were allowed at least two weeks additional acclimatization before transfer to testing containers and introduction of Treflan E.C. Suggestions by Brett (1944) and Allen (1970) on acclimatizing fishes were followed. Fish transferred from acclimatizing aquaria to test containers were done so with great care. Only small mesh dip nets or wet hands that would not remove external mucosa were used to quickly transfer the fishes to test conditions. Any fish that was mishandled, accidentally dropped, or behaving differently from others was discarded. Incidence of disease or deaths among control

fishes that were acclimatized was less than 5% with all groups of fishes tested.

During 96 hour bioassays and recovery periods, the number of dead fish in each container were observed and recorded at 24 hour intervals. Dead fish were noted. The criteria employed to evaluate responses of fishes by Lennon and Walker (1964) served as a very helpful guide.

Water used in the bioassay was taken from the John G. Shedd Aquarium's 250,000 gallon ambient fresh water reservoirs. This water is pumped into the Aquarium from Lake Michigan. Water quality determinations provided by the Chicago Water Purification Plant are applicable to the Aquarium's water supply (Table 1). Carbon dioxide was determined with a Hach Chemical Company (Ames, Iowa) water analysis test kit. Ammonia nitrogen was determined using Hach Chemical Company colorimetric procedures and test kits calibrated for use with the Bausch and Lomb Spectronic 20. A model 54BP oxygen meter, manufactured by the Yellow Springs Instrument Company (Yellow Springs, Ohio) was used to monitor the dissolved oxygen content. The dissolved oxygen was maintained at between 7.0 and 7.8 ppm during bioassays and recovery periods.

Preliminary investigations warranted modifications of static bioassay procedures. The modifications were artificial oxygenation and renewal of test solutions. They were employed according to American Public Health Association Standard Methods (1971). Artificial areation was necessary to maintain control deaths under 10%. Surfacing and gulping of air did not take place by controls when aeration was 7.0 to 7.8 ppm. During preliminary investigations where water was not renewed at twenty-four periods, an accumulation of carbon dioxide and ammonia nitrogen was determined (Table 6).

These are metabolic products; carbon dioxide is a respiratory waste product while of the nitrogen products excreted by fish, ammonia accounts for 25-50%, [the remainder being urea, creatine, amino acids and others (Baldwin, 1949)] to which fish are highly sensitive (Saeki, 1964). Accumulation of metabolic products could cause an increase in toxicity (Doudoroff et al., 1951). Renewal of test solutions also guards against depletion of herbicide due to degradation, volatility, combination with metabolic products or mucus of test animals, and reaction with dissolved substances or testing materials. Renewal maintains a more consistent level of herbicide while limiting interactions of the herbicide or synergisms caused by accumulation of metabolic wastes.

Table 1. CITY OF CHICAGO, DEPARTMENT OF WATER & SEWERS, BUREAU OF WATER
WATER PURIFICATION DIVISION, WATER PURIFICATION LABORATORY, CHEMISTRY UNIT
CENTRAL & NORTH WATER DISTRICT SEASONAL REPORT

SAMPLES COLLECTED: March 28, 1972 ANALYSIS COMPLETED: April 12, 1972

PARAMETER	RAW SHORE	OUTLET	DISTRIBUTION CENTRAL	DISTRIBUTION NORTH
TEMPERATURE, °F *	40	40	40	40
TURBIDITY, JTU *	8	0.2	0.3	0.3
ODOR, STRAIGHT *	2M	4Cc	3M	4Cc
ODOR, DECHLORINATED	-	1DM	-	2M
COLOR *	4	0	0	0
pH *	8.28	8.19	8.18	8.12
ALKALINITY, PHTH	0	0	0	0
ALKALINITY, TOTAL	111	111	110	108
OXYGEN, DISSOLVED	12.9	12.9	12.9	12.9
OXYGEN DEMAND (COD)	10.3	8.2	7.5	8.5
NITROGEN, ORGANIC	0.104	0.048	0.032	0.032
NITROGEN, AMMONIA	0.00	0.11	0.10	0.10
NITROGEN, NITRATE	0.27	0.32	0.27	0.33
NITROGEN, NITRITE	0.004	0.002	0.002	0.003
PHENOL	0.003	0.004	0.006	0.003
DETERGENT (MBAS)	ND	ND	ND	ND
RESIDUE, FILTRABLE	174	180	180	181
RESIDUE, TOTAL	187	183	185	186
RESIDUE, TOTAL FIXED	111	89	106	91
SILICA (SiO_2)	1.43	1.18	1.60	1.60
SULFATE (SO_4)	19.0	20.0	23.3	23.8
PHOSPHATE (PO_4)	0.09	0.05	0.03	0.03
ALUMINUM	0.08	0.08	0.08	0.09
ARSENIC	ND	ND	ND	ND
BORON	ND	ND	ND	ND
CADMIUM	0.001	0.001	0.001	ND
CHROMIUM VI	ND	ND	ND	ND
CHROMIUM, TOTAL	ND	ND	0.008	0.010
COBALT	0.006	0.004	0.009	0.006
COPPER	0.001	0.003	0.004	0.001
IRON, SOLUBLE	0.01	0.01	0.02	0.02
LEAD	ND	ND	ND	ND
LITHIUM	0.008	0.002	0.001	0.002
MANGANESE	0.005	0.004	0.003	0.002
MERCURY, ppb *	<0.1	<0.1	<0.1	<0.1
NICKEL	0.001	0.001	0.001	ND
ZINC	0.002	ND	0.012	0.020
CALCIUM	36	36	37	36
MAGNESIUM	11	10	10	11
POTASSIUM	0.86	1.5	1.7	0.86
SODIUM	4.4	5.3	5.3	5.3
STRONTIUM	0.04	0.04	0.04	0.04
RADIOACTIVITY, pc/l *	2.5 ± 0.5	1.8 ± 0.4	1.7 ± 0.4	1.5 ± 0.4
CONDUCTIVITY, umhos/cc *	275	295	285	295
CHLORIDE	9.6	12.5	13.1	13.0
CYANIDE	ND	ND	ND	ND
FLUORIDE	0.12	0.92	0.93	0.98
BICARBONATE as $CaCO_3$	109	109	108	107
CARBONATE as $CaCO_3$	2.33	1.92	1.83	1.58
HARDNESS as $CaCO_3$	136	133	134	134
(L) STABILITY INDEX	- 0.01	- 0.10	- 0.12	- 0.18

* All other results expressed in ppm

RESULTS

The following material represents data accumulated during the bioassays involving twenty species of fish subjected to the herbicide Treflan E.C. These data will be discussed in the Conclusion.

Table 2. Taxonomic Information (Greenwood et al. 1966), Common Names, Average Size and Weight of Bioassay Fishes.

Taxonomic Information	Common Name	Average Size (inches)	Average Weight (grams)
Order Cypriniformes			
Suborder Characoidei			
Family Characidae			
Ctenobrycon spilurus	Silver Tetra	2 3/4	5.0
Hemigrammus ocellifer	Head-and-tail-light Tetra	1 3/8	1.0
Hemigrammus rhodostomus	Runny Nose Tetra	1 3/8	1.0
Suborder Cyprinoidei			
Family Cyprinidae			
Barbus conchonius	Rosy Barb	2 3/4	5.6
Barbus filamentosus	Black-spot Barb	4 1/4	12.2
Carassius auratus	Gold fish	2	3.0
Notemigonus crysoleucas	Golden Shiner	3 3/4	10.0
Pimephales promelas	Fathead Minnow	2 1/4	1.5
Order Siluriformes			
Family Ictaluridae			
Ictalurus punctatus	Channel Catfish	4	9.5
Family Callichthyidae			
Corydoras aeneus	Bronze Cat	1	0.5

(Table continued next page)

Table 2. (continued)

Taxonomic Information	Common Name	Average Size (inches)	Average Weight (grams)
Order Atherinomorpha			
Suborder Cyprinodontoidei			
Family Poeciliidae			
Lebistes reticulatus	Guppy	3/4	0.6
Mollienisia latipinna	Perma-black Molly	1 1/2	1.4
Xiphophorus helleri	Green Swordtail	2 3/8	3.0
Xiphophorus maculatus	Blue Platy	1 1/2	0.7
Xiphophorus variatus	Sunset Variatus	1 1/2	0.8
Order Perciformes			
Suborder Percoidei			
Family Centrarchidae			
Lepomis macrochirus	Bluegill	3	8.2
Family Cichlidae			
Cichlasoma centrarchus	Centrarchus	1 3/4	3
Haplochromis burtoni	Burtoni	3 1/2	9.5
Pseudotropheus auratus	Auratus	2 1/4	1.6
Suborder Anabantoidei			
Family Anabantidae			
Trichogaster tichopterus	Three-spot Gourami	2 1/2	3.5

Table 3. Toxicity of Treflan E.C. at 0.75, 1.275 and 1.8 ppm on twenty species of fish.

Specimens (Scientific Name)	Concentration of Treflan ppm	Number of Fish	Total Number Dead*@ 24 hr	48 hr	72 hr	96 hr
Family Characidae						
Ctenobrycon spilurus	0.75	24	0	0	0	0
" "	1.275	24	0	0	0	0
" "	1.8	24	0	0	0	0
Hemigrammus ocellifer	0.75	18	0	0	4	6
" "	1.275	12	0	0	3	9
Hemigrammus rhodostomus	0.75	20	2	12	18	20
" "	1.275	15	4	13	15	15
Family Cyprinidae						
Barbus conchonius	0.75	12	0	0	0	0
" "	1.275	12	1	2	2	2
" "	1.8	12	0	0	0	5
Barbus filamentosus	0.75	30	0	0	0	0
" "	1.275	30	2	4	4	4
" "	1.8	20	0	0	0	7

*mortalities are cumulative

(continued next page)

Table 3. (continued)

Specimens (Scientific Name)	Concentration of Treflan ppm	Number of fish	Total Number Dead* @ 24 hr	48 hr	72 hr	96 hr
Family Cyprinidae (continued)						
Carassius auratus	0.75	40	0	2	2	2
" "	1.275	40	0	4	6	10
" "	1.8	40	0	4	16	24
Notemigonus crysoleucas	0.75	24	0	8	20	24
" "	1.275	24	0	3	20	24
" "	1.8	24	0	14	24	24
Pimephales promelas	0.75	36	14	19	28	36
" "	1.275	36	8	14	32	36
" "	1.8	36	12	27	36	36
Family Ictaluridae						
Ictalurus punctatus	0.75	80	0	0	0	0
" "	1.275	80	0	0	0	0
" "	1.8	40	0	0	2	16
Family Callichthyidae						
Corydoras aeneus	0.75	12	0	0	0	0
" "	1.275	12	0	0	0	1
" "	1.8	12	0	0	0	1

*mortalities are cumulative

(continued next page)

Table 3. (continued)

Specimens (Scientific Name)	Concentration of Treflan ppm	Number of Fish	Total Number Dead* @ 24 hr	48 hr	72 hr	96 hr
Family Poeciliidae						
Lebistes reticulatus	0.75	36	4	6	12	14
" "	1.275	36	0	0	6	9
" "	1.8	24	2	6	6	6
Mollienisia latipinna	0.75	15	0	0	0	2
" "	1.275	15	0	0	0	5
" "	1.8	15	3	3	3	6
Xiphophorus helleri	0.75	18	0	0	0	2
" "	1.275	18	1	1	3	3
" "	1.8	18	2	3	5	11
Xiphophorus maculatus	0.75	18	0	0	0	6
" "	1.275	18	4	5	5	9
" "	1.8	18	4	5	5	13
Xiphophorus variatus	0.75	18	0	1	4	8
" "	1.275	18	0	0	0	2
" "	1.8	18	0	2	6	12

*mortalities are cumulative

(continued next page)

Table 3. (continued)

Specimens (Scientific Name)	Concentration of Treflan ppm	Number of Fish	Total Number Dead* @ 24 hr	48 hr	72 hr	96 hr
Family Centrarchidae						
Lepomis macrochirus	0.75	50	0	0	2	8
" "	1.275	50	0	17	20	28
" "	1.8	25	0	18	20	25
Family Cichlidae						
Cichlasoma centrarchus	0.75	20	0	0	0	0
" "	1.275	20	0	0	0	0
" "	1.8	12	0	0	0	0
Haplochromis burtoni	0.75	12	0	0	0	0
" "	1.275	12	0	0	0	1
Pseudotropheus auratus	0.75	18	0	0	0	1
" "	1.275	18	11	12	13	13
Family Anabantidae						
Trichogaster trichopterus	0.75	18	0	8	8	12
" "	1.275	12	0	9	9	9
" "	1.8	18	0	14	14	14

*mortalities are cumulative

Table 4. Recovery of fishes exposed to Treflan E.C. Deaths are cumulative.

Specimens (Scientific Name)	Exposure to Treflan ppm	Number of Fish	Deaths at 96 hour Exposure	Number of Fish in Recovery Tanks	Cumulative Mortalities 240 hr	480 hr
Family Characidae						
Ctenobrycon spilurus	0.75	24	0	24	0	0
" "	1.275	24	0	24	0	0
" "	1.8	24	0	24	10	10
Hemigrammus ocellifer	0.75	18	6	12	12	12
" "	1.275	12	9	3	3	3
Hemigrammus rhodostomus	0.75	20	20	0	--	--
" "	1.275	15	15	0	--	--
Family Cyprinidae						
Barbus conchonius	0.75	12	0	12	2	4
" "	1.275	12	2	10	6	8
" "	1.8	12	5	7	0	4
Barbus filamentosus	0.75	30	0	30	4	12
" "	1.275	30	4	26	18	22
" "	1.8	20	7	13	0	11

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Table 4. (continued)

Specimens (Scientific Name)	Exposure to Treflan ppm	Number of Fish	Deaths at 96 hour Exposure	Number of Fish in Recovery Tanks	Cumulative Mortalities 240 hr	480 hr
Family Poeciliidae						
Lebistes reticulatus	0.75	36	14	22	7	7
" "	1.275	36	9	27	14	16
" "	1.8	24	6	18	14	15
Mollienisia latipinna	0.75	15	2	13	4	4
" "	1.275	15	5	10	5	8
" "	1.8	15	6	9	6	6
Xiphophorus helleri	0.75	18	2	16	11	16
" "	1.275	18	3	15	14	15
" "	1.8	18	11	7	7	7
Xiphophorus maculatus	0.75	18	6	12	12	12
" "	1.275	18	9	9	9	9
" "	1.8	18	13	5	5	5
Xiphophrus variatus	0.75	18	8	10	4	4
" "	1.275	18	2	16	9	9
" "	1.8	18	12	6	4	5

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Table 4. (continued)

Specimens (Scientific Name)	Exposure to Treflan ppm	Number of Fish	Deaths at 96 hour Exposure	Number of Fish in Recovery Tanks	Cumulative Mortalities 240 hr	480 hr
Family Cyprinidae (continued)						
Carassius auratus	0.75	40	2	38	10	12
" "	1.275	40	10	30	20	25
" "	1.8	40	24	16	16	16
Notemigonus crysoleucas	0.75	24	24	0	--	--
" "	1.275	24	24	0	--	--
" "	1.8	24	24	0	--	--
Pimephales promelas	0.75	36	36	0	--	--
" "	1.275	36	36	0	--	--
" "	1.8	36	36	0	--	--
Family Ictaluridae						
Ictalurus punctatus	0.75	80	0	80	0	0
" "	1.275	80	0	80	18	40
" "	1.8	40	16	24	24	24
Family Callichthyidae						
Corydoras aeneus	0.75	12	0	12	1	1
" "	1.275	12	1	11	3	4
" "	1.8	12	1	11	5	6

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Table 4. (continued)

Specimens (Scientific Name)	Exposure to Treflan ppm	Number of Fish	Deaths at 96 hour Exposure	Number of Fish in Recovery Tanks	Cumulative Mortalities 240 hr	480 hr
Family Centrarchidae						
Lepomis macrochirus	0.75	50	8	42	42	42
" "	1.275	50	28	22	22	22
" "	1.8	25	25	0	--	--
Family Cichlidae						
Cichlasoma centrarchus	0.75	20	0	20	0	0
" "	1.275	20	0	20	1	1
" "	1.8	12	0	12	0	0
Haplochromis burtoni	0.75	12	0	12	0	0
" "	1.275	12	1	11	0	0
Pseudotropheus auratus	0.75	18	1	17	0	1
" "	1.275	18	13	5	0	0
Family Anabantidae						
Trichogaster trichopterus	0.75	18	12	6	4	3
" "	1.275	12	9	3	3	3
" "	1.8	18	14	4	4	4

Table 5. Effects of Treflan E.C. on Equilibrium.

Specimens (Scientific Name)	Concentration of Treflan ppm	No. of Fish	No. of Fish with Equilibrium / Total No. of Fish 24 hr	48 hr	72 hr	96 hr	No. of Fish in Recovery Tanks	240 hr	480 hr
Family Characidae									
Ctenobrycon spilurus	0.75	24	22/24	22/24	22/24	22/24	24	24/24	24/24
" "	1.275	24	24/24	24/24	24/24	22/24	24	24/24	24/24
" "	1.8	24	24/24	22/24	22/24	22/24	24	14/14	14/14
Hemigrammus ocellifer	0.75	18	12/18	6/18	6/14	4/12	12	0/0	0/0
" "	1.275	12	3/12	3/12	3/9	0/3	3	0/0	0/0
Hemigrammus rhodostomus	0.75	20	9/18	3/8	2/2	0/0	0	----	----
" "	1.275	15	4/11	0/2	0/0	0/0	0	----	----
Family Cyprinidae									
Barbus conchonius	0.75	12	7/12	3/12	3/12	3/12	12	4/10	8/8
" "	1.275	12	1/11	0/10	0/10	0/10	10	1/4	1/2
" "	1.8	12	4/12	0/12	0/12	0/7	7	0/7	0/3
Barbus filamentosus	0.75	30	14/30	8/30	6/30	6/30	30	12/26	18/18
" "	1.275	30	2/28	0/26	0/26	0/26	26	2/8	3/4
" "	1.8	20	7/20	0/20	0/20	0/13	13	0/13	0/2

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Table 5. Effects of Treflan E.C. on Equilibrium. (continued)

Specimens (Scientific Name)	Concentration of Treflan ppm	No. of Fish	No. of Fish with Equilibrium / Total No. of Fish 24 hr	48 hr	72 hr	96 hr	No. of Fish in Recovery Tanks	240 hr	480 hr
Family Cyprinidae (continued)									
Carassius auratus	0.75	40	40/40	38/38	36/38	32/38	38	28/28	26/26
" "	1.275	40	36/40	28/36	24/34	20/30	30	4/10	5/5
" "	1.8	40	32/40	32/36	24/24	16/16	16	0/0	0/0
Notemigonus crysoleucas	0.75	24	8/24	6/16	0/4	0/0	0	----	----
" "	1.275	24	10/24	5/21	4/4	0/0	0	----	----
" "	1.8	24	11/24	3/10	0/0	0/0	0	----	----
Pimephales promelas	0.75	36	15/22	13/17	3/8	0/0	0	----	----
" "	1.275	36	18/28	8/22	2/4	0/0	0	----	----
" "	1.8	36	11/24	0/9	0/0	0/0	0	----	----
Family Ictaluridae									
Ictalurus punctatus	0.75	80	80/80	80/80	80/80	80/80	80	80/80	80/80
" "	1.275	80	80/80	80/80	80/80	78/80	80	53/62	40/40
" "	1.8	40	40/40	40/40	38/38	24/24	24	0/0	0/0
Family Callichthyidae									
Corydoras aeneus	0.75	12	11/12	11/12	11/12	12/12	12	11/11	11/11
" "	1.275	12	11/12	8/12	9/12	9/11	11	8/8	7/7
" "	1.8	12	11/12	7/12	7/12	11/12	11	6/6	5/5

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Table 5. Effects of Treflan E.C. on Equilibrium. (continued)

Specimens (Scientific Name)	Concentration of Treflan ppm	No. of Fish	No. of Fish with Equilibrium / Total No. of Fish 24 hr	48 hr	72 hr	96 hr	No. of Fish in Recovery Tanks	240 hr	480 hr
Family Poeciliidae									
Lebistes reticulatus	0.75	36	32/32	17/30	20/24	18/22	22	15/15	15/15
" "	1.275	36	35/36	30/36	25/30	17/27	27	13/13	11/11
" "	1.8	24	18/22	12/18	14/18	14/18	18	4/4	3/3
Mollienisia latipinna	0.75	15	15/15	14/15	14/15	11/13	13	9/9	9/9
" "	1.275	15	15/15	8/15	10/15	5/10	10	3/5	2/2
" "	1.8	15	11/12	11/12	11/12	4/9	9	3/3	3/3
Xiphophorus helleri	0.75	18	16/18	15/18	9/18	11/16	16	3/5	0/0
" "	1.275	18	8/17	7/17	8/15	7/15	15	1/1	0/0
" "	1.8	18	8/16	5/15	6/13	5/7	7	0/0	0/0
Xiphophorus maculatus	0.75	18	14/18	14/18	10/18	0/12	12	0/0	0/0
" "	1.275	18	7/14	4/13	7/13	4/9	9	0/0	0/0
" "	1.8	18	12/14	6/13	10/13	2/5	5	0/0	0/0
Xiphophorus variatus	0.75	18	18/18	13/17	9/14	6/10	10	6/6	6/6
" "	1.275	18	4/18	4/18	3/18	5/16	16	7/7	7/7
" "	1.8	18	9/18.	9/16	5/12	2/6	6	2/2	1/1

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Table 5. Effects of Treflan E.C. on Equilibrium. (continued)

Specimens (Scientific Name)	Concentration of Treflan ppm	No. of Fish	No. of Fish with Equilibrium / Total No. of Fish 24 hr	48 hr	72 hr	96 hr	No. of Fish in Recovery Tanks	240 hr	480 hr
Family Centrarchidae									
Lepomis macrochirus	0.75	50	8/50	0/50	0/48	0/42	42	0/0	0/0
" "	1.275	50	5/50	0/33	0/30	0/22	22	0/0	0/0
" "	1.8	25	3/25	0/7	0/5	0/0	0	----	----
Family Cichlidae									
Cichlosoma centrarchus	0.75	20	20/20	19/30	13/20	13/20	20	20/20	20/20
" "	1.275	20	12/20	5/20	4/20	0/20	20	16/19	19/19
" "	1.8	12	6/12	0/12	0/12	0/12	12	6/12	12/12
Haplochromis burtoni	0.75	12	11/12	8/12	8/12	8/12	12	12/12	12/12
" "	1.275	12	6/12	5/12	6/12	6/11	11	10/11	11/11
Pseudotropheus auratus	0.75	18	16/18	14/18	13/18	11/17	17	17/17	16/16
" "	1.275	18	7/7	6/6	5/5	5/5	5	2/5	5/5
Family Anabantidae									
Trichogaster trichopterus	0.75	18	10/18	4/10	2/10	2/6	6	4/4	3/3
" "	1.275	12	9/12	0/3	1/3	1/3	3	3/3	3/3
" "	1.8	18	13/18	2/6	2/6	2/4	4	4/4	4/4

Table 6. Build-up of ammonia and carbon dioxide in preliminary bioassays where water was not renewed.

	0 hours	24 hours	48 hours	72 hours	96 hours
Carbon dioxide (ppm)	5.0	15.0	15.0	15.0	15.0
Ammonia nitrogen (ppm)	.30	.50	1.0	1.17	1.50

DISCUSSION

Many of the following observations are subjective, but represent individual interpretations of the general effects fishes can exhibit when exposed to Treflan E.C. at a toxic level.

Behavior

A pronounced difference between fishes being tested and those under control conditions could be observed within two hours after the addition of Treflan E.C. The fishes were irritable and excitable. Respiration became increased and irregular as estimated by gill movement. Within twenty four hours many of the fishes were motionless against the sides or on the bottom of the container. Many fishes exhibited a lack of equilibrium (Table 5) and were lying on their sides. A few became inverted or floated on their sides. Fishes in family Poeciliidae had difficulty regaining equilibrium while Cichlids, particularly two genera, Haplochromis and Cichlasoma, exhibited an efficiency of recovery, with all afflicted fish recovering. In most situations, fishes that did not regain equilibrium by the termination of the twenty day recovery period died. An exception to this were fishes of the genus Barbus, family Cyprinidae. (A few Barbus did not regain equilibrium but were living after twenty days of the recovery period. Death occurred within thirty days of the recovery period.)

Irritability was tested by subjecting the fishes to a stimulus such as tapping on the container or probing with a net. Commonly they became excitable and responded by swimming away from the stimulus in an erratic, skittering, convulsive manner. In most cases, mobility was limited to short erratic spurts about the container during the 96 hours of exposure. At lesser concentrations of herbicide, many fishes that maintained equilibrium also maintained swimming ability and seemed unaffected in these capacities. However, these fishes were lethargic, exhibited respiratory difficulties and were extremely irritable to stimuli. A hurried movement away from the slightest tapping or probing characterized the response of these fish.

Loss of equilibrium, orientation, excessive excitability and convulsive movements indicate Treflan E.C. affects the central nervous system. The effects of Treflan E.C. on the central nervous system could also be related to the increased and irregular respiration the fishes exhibited. Excitability, irritability and hurried responses to stimuli could cause an increase in metabolism resulting in respiratory irregularities and increases.

External Anatomy

The integument of the fishes in most cases showed light discolorations. However, Cichlasoma centrarchus and Lepomis macrochirus also exhibited dark discoloration and varidiscoloration. The external mucosa underwent no observable changes. In some cases the integument was hemorrhagic. A scoliosis characterized by abrupt curvature of the spine and body flexure

was observed in many specimens. This reaction is similar to the response of fishes undergoing chronic exposure to sublethal levels of organophosphate insecticides. Organophosphate insecticide exposure frequently is followed by a scoliosis characterized by an abrupt curvature of the spine in the area just posterior to the rib cage. Many fish develop a compensatory flexure to bring the spine back in line with the longitudinal axis of the body. If exposure is prolonged, the curvature becomes permanent (Meyer, 1966). Additional investigations would be necessary to determine the cause of the scoliosis.

Hematoma, a swelling filled with extravasated blood, occurred in varying degrees and in varying positions on the fishes. The position and amount of hematoma and scoliosis were so variable interspecifically as well as intraspecifically, they were not recorded as a specific determination. The phenomena of hematoma and scoliosis is mentioned in specific observations recorded in family observations which follow. Body flexure and curvature of the spine causing pressure on the dorsal aorta and other blood vessels is the probable cause of hematoma. All fishes examined showed rigor mortis in death.

Family Characidae

Ctenobrycon spilurus, described by Innes (1966) as a hardy fish, Hemigrammus ocellifer, somewhat less hardy, and Hemigrammus rhodostomus, a somewhat sensitive species (Sterba, 1969), represented the family Characidae. The tolerance of these fishes to Treflan E.C. was reflective to their general hardiness. This held true for all species tested. C. spilurus exhibited the greatest tolerance to the toxicant by Characids.

LC_0 for bioassays at all concentrations prescribed was recorded. All C. spilurus which lost equilibrium at 0.75 ppm and 1.275 ppm regained it during the recovery period. During recovery after exposure at 1.8 ppm, some mortalities occurred (Table 4). Hemorrhagic integument and body flexure characterized by curvature of the spine was observed in some fish which later died. H. ocellifer showed greater resistance to Treflan than H. rhodostomus. Although some H. ocellifer survived the 96 hour toxicity experiments, none survived the recovery period. H. rhodostomus did not survive the 96 hour bioassay. A LC_{100} was established at 96 hours for 0.75 ppm and 72 hours for 1.275 ppm. Loss of equilibrium also occurred at an increased rate in H. rhodostomus (Table 5). Some hemorrhaging was observed in both species of Hemigrammus. Body flexure was slight. Characidae have a lower vertebral number than fishes in any of the other families tested (Greenwood et al., 1966). A lower vertebral number offers a possible explanation for the occurrence of slight scoliosis as opposed to greater scoliosis with a greater vertebral number.

Family Cyprinidae

Five species representing the Family Cyprinidae were exposed to Treflan E.C. Barbus conchonius and Barbus filamentosus showed a LC_0 at 0.75 ppm for the 96 hour bioassay. Both species suffered deaths during recovery. As shown in Table 5, many fish which lost equilibrium regained it during recovery following exposure to 0.75 ppm Treflan E.C. At increased concentrations of herbicide both species showed considerable losses with the majority occurring during recovery. As is generally the case fishes exhibited greater and earlier losses of equilibrium at higher

concentrations of herbicide. The percentage of mortalities occurring in Carassius auratus were very similar to those occurring in both species of Barbus. In C. auratus a LC_{100} was reached after ten days of recovery at 1.8 ppm. No LC_{100} occurred with either species of Barbus at 1.8 ppm during the bioassay or twenty days of recovery, although all survivors were lying on their sides and exhibited equilibrium loss. LC_{100}s occurred within thirty days of recovery with both species of Barbus. Pimephales promelas and Notemigonus crysoleucas had LC_{100}s after 96 hours of exposure to Treflan E.C. at 0.75 ppm and 1.275 ppm. At 1.8 ppm both species showed LC_{100}s for 72 hours of exposure.

In the family Cyprinidae all species exhibited scoliosis. In the C. auratus scoliosis occurred at a lesser degree and no hematoma was observed. Chronic scoliosis resulted in deformities. Hematoma was observed in all other Cyprinids tested. It was most pronounced and occurred more frequently with Pimephales promelas.

Family Ictaluridae

Ictalurus punctatus was the only species representing family Ictaluridae. Treflan E.C. had no apparent affect at 0.75 ppm. A LC_0 was recorded after 96 hours of exposure at 1.275 ppm. Loss of equilibrium occurred at 1.275 ppm with the majority observed during recovery. A LC_{50} was established following twenty days of recovery time. Treflan E.C. at 1.8 ppm caused a LC_{40} after 96 hours. A LC_{100} occurred within ten days of recovery time. "Normal" equilibrium loss with the fish lying on their sides did not occur at 1.8 ppm. However, many fish when erratically attempting movement would turn on their sides. Hematoma was not apparent

but curvature of the spine was acute in some cases. Curvature of the spine occurring posterior to the dorsal fin was most pronounced in these channel catfish. Deformities were the result of some fish exhibiting the curvature.

Family Callichthyidae

Corydoras aeneus were used as representatives of this family. During the 96 hour bioassay almost no deaths occurred at all concentrations tested. Mortalities at about 50% occurred following the recovery period after exposure at 1.275 and 1.8 ppm. Some C. aeneus were observed without equilibrium (on their sides). Curvature of the spine and hematoma was not apparent in this species.

Family Poeciliidae

Five species represented the family Poeciliidae. Response to the herbicide reflects the species' general hardiness as expressed by Innes (1966), and Sterba (1969). Xiphophorus helleri and X. maculatus both showed LC_{100}s at all concentrations by the termination of the recovery period. In both fishes the greatest mortality during 0.75 and 1.275 dosages occurred during the recovery period. At a concentration of 1.8 ppm the majority of the mortalities occurred during the 96 hour exposure period. No LC_{100}s were observed in Xiphophorus variatus, Lebistes reticulatus or Molliensia latipinna. The number of mortalities increased with dosage as expected. Hematoma was observed in Lebistes reticulatus, Xiphophorus maculatus and X. helleri. Body flexure associated with curvature of the spine was present in all Poecilids. It occurred to a lesser degree in X. variatus.

Family Centrarchidae

The bluegill, Lepomis macrochirus was used as a representative of Centrarchids. Centrachidae are generally susceptible to abrupt alterations in environmental conditions (Sterba, 1969), and proved extremely sensitive to Treflan E.C. LC_{100}s were observed following exposure to trifluralin at all concentrations. At 0.75 and 1.275 ppm LC_{100}s occurred by the termination of ten days recovery time. At 1.8 ppm the LC_{100} occurred after 96 hours. All bluegill exposed to Treflan E.C. lost equilibrium rapidly and remained on their sides throughout most of the testing period. Most fish lost equilibrium within 24 hours with all fish tested losing equilibrium by the end of a 48 hour period. No fish were observed regaining equilibrium. Hematoma and scoliosis was observed in some cases.

Family Cichlidae

Cichlasoma centrarchus, Haplochromis burtoni and Pseudotropheus auratus represent the Cichlids. Cichlasoma centrarchus proved to be the most tolerant of all fishes exposed to Treflan E.C. Only one fish was lost during exposure at test concentrations. LC_0s were recorded at concentrations of 0.75 and 1.8 ppm. C. centrarchus also gained distinction by exhibiting its ability to regain equilibrium. As recorded in Table 5, although all fish lost equilibrium at 1.275 and 1.8 ppm, only one fish did not recover, and that one died. Haplochromis burtoni was equally tolerant to Treflan E.C. but fewer fish underwent experimentation. H. burtoni were less susceptible to losing equilibrium and also exhibited great abilities to regain equilibrium (Table 5). Pseudotropheus auratus were the most sensitive of the Cichlids tested. Although many fish were lost after the

first day of exposure, deaths were greatly reduced in following observations. All fish which lost equilibrium regained it by the termination of the recovery period. The strongest concentration tested on P. auratus was 1.275 ppm which resulted in a LC_{72} for 96 hours. No fish died during recovery. Hematoma was not observed in the Cichlidae. Curvature of the spine and compensatory flexure occurred in all species tested. Under chronic exposure body deformities occurred.

Cichlids exhibit remarkable recovery and ability to regain equilibrium. When Cichlids are administered MS-222 (an anesthetic) prior to electromyography they recover within five minutes from a dosage that would require several hours of recovery time for a very hardy fish, such as the carp Cyprinus carpio (Linnaeus), (Liem, 1972). It is suggested that Cichlid's remarkable recovery from Treflan E.C. as well as MS-222 is probably pharmacological and would require extensive research to determine. Anatomical differences are probably not a factor in the Cichlid's ability to regain equilibrium. The Centrarchidae, Cichlidae and Anabantidae are all physoclistous, a condition in which the gas bladder reaches its fullest development as a hydrostatic organ (Lagler et al. 1962). If this was a factor helping the Cichlids regain equilibrium, it was not evident in the other fishes with this condition. There is a slight possibility the lateral line (a sensory system necessary for orientation and equilibrium), which in the Cichlids is divided into a high upper lateral line forward and a low short lateral line toward the rear (Goldstein, 1970), is a factor. Internal investigations of the lateral line show it is quite similar to other fishes.

Family Anabantidae

Trichogaster trichopterus represented the Anabantids. Treflan E.C. caused approximately 75% mortality at all concentrations tested. All deaths during bioassays occurred between 24 and 48 hours of exposure. Recovery data shows few fish recovered following 96 hours of exposure. Scoliosis occurred in many fish. Hematoma was not observed.

CONCLUSION

A pollution biologist determining the affects of a herbicide on the environment would undertake toxicity studies to protect organisms of his concern from damage or death. These studies combined with extensive knowledge of the herbicide in question would dictate its proper usage. By establishing the types of harmful affects exposure to the herbicide at varying concentrations afflicts to bioassay fishes under laboratory conditions, a criteria is established for making hypotheses concerning the herbicide's affects on fishes in natural environments.

It should be made clear that consistency of the response of species of fishes to toxic materials can be influenced by many factors, as demonstrated by Weiss and Botts (1957). Results of independent bioassays often cannot be related because the comparative resistance of many test fishes has not been established (Douglas and Irwin, 1962). Therefore, independent bioassays often present varying data as a result of varying resistance of the fishes due to handling, acclimation, general physiological conditions, water quality and test procedures. An example of such data would be the LC_{50} of 0.59 parts per million for goldfish reported by Elanco Products Incorporated (1968) and a value slightly over 0.75 parts per million determined in this assay. When applying data supplied by a bioassay it should be remembered ~~this~~ these data can be

taken per se for only the exact laboratory procedures described in the experiment.

Variances in the data would be due to changes in conditions. In this study, for example, container type was shown to be a variable. Plastic containers used in preliminary investigations were not used during the bioassay because Treflan E.C. was found to react with plastics causing discoloration. The degree of reaction was not determined. Glass containers meeting all the requirements of the American Public Health Association Standard Methods (1971) were used. They were cleaned, as described in Materials and Methods, to remove all toxic residues that could affect the bioassay data.

Treflan E.C. was a powerful toxicant to the fishes tested. The sensitivities to Treflan E.C. varied considerably. Recovery following exposure to Treflan E.C. was almost irreversible in most families with an exception being Cichlidae. The suggestion by Elanco Products researchers Parka and Worth (1965) that Treflan E.C. used according to label direction presents no hazard to fish, is particularly valid when considering the degradation and decomposition properties of trifluralin, along with its behavior in the soil and during static soil tests. Negligent aerial incorporation, over incorporation, improper disposal, or vapor emission under certain atmospheric conditions could present a definite hazard to fishes. Spills or accidents allowing Treflan E.C. to enter water systems could be very harmful.

Further investigations on the affects of Treflan at different life stages, on additional species, in waters of various chemistries, on minimum lethal concentrations, and warm and cold water, would provide additional data necessary for a complete investigation of the effects of Treflan E.C. on fishes.

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